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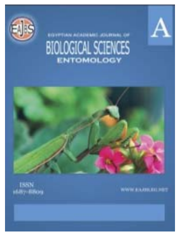

Efficiency of gamma radiation and ozonation against *Sitotroga cerealella* stages (Gelechiidae:Lepidoptera) infesting stored wheat grains and their effect on wheat quality

Abd El-Aziz E. Abd El-Aziz; Hassan, B. H. Hussain and Rasha, A. Zinhoum
Stored product pest Dept., Plant Protection Research Institute, A.R.C., Dokki, Giza, Egypt

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ABSTRACT

The present studies were carried out to determine the efficacy of gamma radiation and ozone technology as management tools to control and kill all life stages of *S. cerealella* (eggs, larvae and pupae) infesting stored wheat grains and their effect on viability and backing characters. The obtained results revealed that *S. cerealella* eggs were slightly tolerant to gamma radiation followed by pupae and larvae stages. It was showed that, the complete reduction in adult emergence (100 %) required 1500 Gy dose for all tested stages. Our results appeared that dose of 1000 Gy or less was inadequate to killing *S. cerealella* eggs and pupae. Also, ozone gas data reported same trend where egg stage was more tolerant to ozone followed pupae and larvae stages. It was the maximum reduction % in adult emergence 69.67 % for eggs followed by 78.00 and 86.11% for pupae and larvae stages at 8 h exposure period. The results appear ozonated wheat grains do not change the quality of baking bread and improved viability of wheat grains. The obtained results demonstrated that the LD_{95} of gamma radiation were 1630, 840 and 1550 Gy for eggs, larvae and pupae stages, resp. These findings for LT_{95} values of ozone for different stages were 33.08, 17.45 and 25.24 hrs for eggs, larvae and pupae stages, resp.

INTRODUCTION

Angoumois grain moth, *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera) has been recognized as a common pest of grains world-wide. Its larvae feed on corn grains causing direct damage (Weston *et al*., 1993). Such damage leads to considerable quantitative and qualitative losses represented as weight loss, decrease of the nutritional value of the grains and failure of grain germination.

Fumigation by methyl bromide is a halogenated compound and it suspected to be carcinogenic, Taylor (1994). The use of methyl bromide has been banned in Egypt since 2015 because of its ozone depleting properties. Fumigation with phosphine is widely used to control stored product pests. It has high toxicity and most effective without deleterious effects on the viability of dormant grain. However, only the continuous and indiscriminate use of phosphine has resulted in the evolution of resistant populations of targeted pests, Lorini *et al.,* (2007) Gamma irradiation may be an effective and economically feasible alternative method for insect disinfestation (Tilton *et al.,* 1978). Most insects can be controlled with doses of less than 0.3 k Gy but some stored-product moths may require doses as high as 1 kGy (Follett and Neven, 2006).

Ozone is highly reactive and a strong oxidizing agent, and is classified as "GRAS" (Generally Recognized As Safe) by (US-EPA) throughout the world. Ozone has been used to purify drinking water, kill bacteria, sanitize food, deodorize, and decrease aflatoxin contamination (Geovana, 2014). Ozone has also shown potential for control of insect strains that are resistant to phosphine (Sousa *et al* 2008).The present studies were carried out to determine efficiency of ozonation and gamma radiation against eggs, larvae and pupae of *Sitotroga cerealella* infesting stored wheat grains and their effect on viability and backing characters

MATERIALS AND METHODS

1- Insect cultures:

Stocks of the insect used in these experiments, Angoumois grain moth, *Sitotroga cerealella* (Oliv.) (Gelechiidae: Lepidoptera) were maintained at stored grain and product pests Department, Plant Protection Research Institute, whereas it was reared at 28 ±2 ^{0}C and 65 ±5 R.H. on whole wheat for at least two months.

2 - Production of ozone gas:

Ozone gas was product from air using an ozone generator Model OZO 6 VTTL OZO Max Ltd, Shefford, Quebec Canada (OZO Max Ltd, Shefford, Quebec, Canada) from purified extra dry oxygen feed gas at the laboratory of Food Toxicology & Contaminants, National Research Center. The amount of ozone output was controlled by a monitor- controller having a plug-in sensor on board which is changed for different ranges of ozone concentration and a belt pan in the monitor-controller allows controlling the concentration in a selected range.

3- Radiation source

Gamma cell radiation Unit 220; (Gy/s) located at the National Center for radiation Research and Technology, Atomic Energy Authority, Nasr City, Cairo, Egypt.

4 - Efficacy of gamma radiation and ozone application as *S. cerealella* disinfestation

Gemmiza11 wheat variety used in this study because, it was the most preferable for this pest Hussain and Nasr (2015).

The experiments were started using small jute bags (about 0.5 kg). Each bag contained 10 g of gemmiza11 wheat artificially infested with twenty five larvae (0-1 day old) of *S. cerealella*. The bags were directly closed well and exposed to different dose of gamma radiation (63, 125, 250, 500, 1000 and 1500 Gy) and 500 ppm of ozone at different exposure periods (1, 2, 4, 6 and 8 hours). Larvae and pupae were exposed to gamma radiation and ozone after 18 and 24 days, respectively. Wheat grains of each bag were carefully transferred into glass jars (7cm diam. and 12cm length) covered with muslin and kept under experimental condition (25 ± 2^{0}C and with 70% RH) till the emergency. Whereas in case of the egg treatments were done through two methods, the eggs alone and eggs with wheat grains to record hatchability %. Three untreated bags were kept as control. All treatments and the control were replicated three times. After four weeks, jars were examined daily to record adult emergence until the emergence of adult stopped. The reduction % of adult emergence was calculated according to the formula of Henderson and Tilton (1955).

$$\text{Reduction \%} = \frac{\text{Control} - \text{treated}}{\text{Control}} \text{ x } 100$$

5. Quality analysis of wheat flour:

5.1. Baking test:

To determine effect of radiation and O_3 on quality of wheat, 2 kg of wheat was exposed to 1500 Gy dose and 8 hours of O_3. After exposure, samples were placed in plastic bags and stored at 4°C until used. Untreated wheat (control) was not exposed to radiation or O_3. 1 kg of wheat from each of (control, O_3 and radiation) was infested with 10 pairs of *S. cerealella* moths for one generation. All wheat samples were baked into toast bread.

Physical aspects of toast bread:

- **Weight:** The average weight (g) of toast bread was determined individually within one hour after baking.
- **Volume:** The volume (cm^3) of different types of produced pan bread was determined by bread volume meter.
- **Specific volume:** calculated according to the method of **(AACC, 2000)** using the equation of:

  Specific volume= Volume (cm^3)/ Weight (g)

Sensory evaluation of toast bread:

The sensory evaluation of toast bread produced (fresh, fresh infestation, ozone, ozone infestation, radiation and radiation infestation) was done as described by **A.A.C.C. (2000**) using ten panelists from the Egyptian Baking Technology Center, Giza. The quality score of toast bread included color (20), texture (20), taste (20), flavor (20), general appearance (20) and overall acceptability was calculated (100). Scores: 90-100 very good (V.G), 80-89 Good (G), 70-79 Satisfactory (S), less than 70 questionably.

6. Statistical analysis:

Data were analyzed using the SPSS computing program using ANOVA, as described by Snedecor and Cochran (1956). Data on the effect of exposure periods on the tested insects were subjected to probit analysis, as described by Finney (1971). LT_{50} and LT_{95} values were calculated using the computer program developed by Noack and Reichmuth (1978).

RESULTS AND DISCUSSION

Results concerning emerged adults and reduction % of progeny produced from artificially infested wheat grains with *S. cerealella* eggs, larvae and pupae treated with gamma radiation are present in **Table (1)**. Emerged adults were 80.29, 49.90, 60.41% (with 20.39, 50.0 and 39.59 % reduction) at 63 Gy dose for egg, larvae and pupae, respectively. However, the complete reduction in emergence (100 % reduction) was obtained at 1500 Gy dose for all tested stages. These results indicate that, eggs stage slightly tolerant to gamma radiation then pupae and larvae stages. Also, the results indicated that, gamma radiation effective in control of this insect. Our results appeared that dose of 1000 Gy or less was inadequate to killing *S. cerealella* eggs and pupae. Similar results were found by **Johnson and Vail (1987),** the dose of radiation required for preventing adult emergence of *P. interpunctella* from treated mature pupae was 92.1 Krad.

Data represented in Table (1) showed that, effects of gamma radiation to produce malformed insects was increased with high ring the doses but completely disappeared at 1500 Gy. Also, the least does (63Gy) not showed any malformation between insect. The other doses produced malformation as 13.1- 46.7, 11.1-33.3 and 3.33-27.8% in cases of treated eggs, larvae and pupae, respectively.

The data obtained in **Table (2)** showed approximately the same trend mentioned before**.** It was noticed that emerged adults % produced of each stage decreased with increasing the exposure period to ozone. In the same time, the least emergence was recorded as 30.70, 13.92 and 22.03 (with 69.67, 86.11 and 78.00 % reduction) for eggs, larvae and pupae respectively, when exposed for the longest period (8h). These results show that egg stages were more tolerant to ozone gas than the two another stages. These results are in agreement with those of Isikber and Öztekin (2009) higher sensitivity for larvae and pupae of *E. kuehniella* and *T. confusum* to ozone when compared to eggs. Also, Hussain (2014) reported that, ozone gas at 5 hours of exposure was sufficient to obtained complete mortality to *Ephestia cautella* larvae.

As for ozone gas, recorded malformation ranged 4.4-9.7, 0.0-11.1 and 0.0-26.9% for treated eggs, larvae and pupae, respectively, Table (2).

Lethal doses of gamma radiation to eggs, larvae and pupae of *S. cerealella* presented in **Table (3)** showed that, the irradiation was more effective in larvae than eggs and pupae stages. LD_{95} values on egg, larvae and pupae of *S. cerealella* were 1630, 840 and 1550 Gy, respectively, agree with the results of Al-Zahaby *et al* (1997) they noticed that the lethal dose for pupae of *E. cautella* was 1000 Gy. Also, in case of ozone, the results reported that mortality % increased with the increasing the exposure time and the larvae were more susceptible than egg and Pupae stages. LT_{95} values on egg, larvae and pupae were 33.08, 17.45 and 25.24 days, respectively Table (4).

Table (1): Emerged adult % and reduction % of progeny of *S. cerealella* irradiated as eggs, larvae and pupae inside wheat grains gamma radiation by different doses of gamma radiation

Dose (GY)	Eggs			Larvae			Pupae		
	Adult emergence %	Reduction %	Malformation %	Adult emergence %	Reduction %	Malformation %	Adult emergence %	Reduction %	Malformation %
63	80.29 ±3.58 a	20.39 ±2.52 e	0 ±0.00b	49.90 ±1.54 a	50.00 ±1.25 d	0 ±0.00c	60.41 ±1.22a	39.59 ±1.22d	0 ±0.00d
125	58.27 ±5.20 b	42.45 ±2.48 d	0 ±0.00b	33.25 ±2.68 b	66.67 ±1.21 c	0 ±0.00c	49.95 ±3.67ab	50.84 ±2.60c	3.33 ±1.34cd
250	38.42 ±2.47 c	61.85 ±2.38 c	0 ±0.00b	21.23 ±2.13c	78.70 ±1.45 b	11.11 ±0.00 b	26.88 ±1.10b	73.85 ±1.10b	13.10 ±1.25c
500	27.27 ±1.46 d	72.81 ±1.15 b	13.13 ±0.42ab	8.34 ±0.13 d	91.67 ±0.00 a	22.22 ±1.12 b	18.37 ±1.47c	82.20 ±1.47ab	19.44 ±1.03b
1000	8.24 ±0.82 e	92.23 ±1.06 a	46.66 ±2.11a	0.95 ±0.95 e	99.07 ±0.93 a	33.33 ±0.00 a	8.04 ±2.21d	91.88 ±1.21a	27.78 ±1.72a
1500	0 ±0.00 e	100 ±0.00a	0 ±0.00b	0 ±0.00 e	100 ±0.00 a	0 ±0.00c	0 ±0.00e	100 ±0.00a	0 ±0.00d
LSD 0.05	11.14	8.19	42.34	7.06	8.35	51.93	12.12	12.32	10.20

Table (2): Emerged adult % and reduction % of progeny of *S. cerealella* exposed as eggs, larvae and pupae inside stored wheat grains to 500 ppm ozone gas at different exposure time (h.)

Exposure Time (h)	**Eggs**			**Larvae**			**Pupae**		
	Adult emergence %	**Reduction %**	**Malformation %**	**Adult emergence %**	**Reduction %**	**Malformation %**	**Adult emergence %**	**Reduction %**	**Malformation %**
1	88.15 ±2.91 [a]	12.56 ±1.70 [d]	0 ±0.00 [a]	84.23 ±1.14 [a]	15.74 ±2.45 [e]	0 ±0.00 [b]	86.32 ±3.48 [a]	13.70±2.48 [d]	0±0.00 [b]
2	64.66 ±1.07 [b]	36.28 ±2.75 [c]	4.41 ±0.04 [a]	60.16 ±1.98 [b]	39.81 ±2.00 [d]	0 ±0.00 [b]	61.74 ±1.94 [b]	38.30±1.65 [c]	0±0.00 [b]
4	46.79 ±2.18 [c]	53.37 ±3.99 [b]	6.48 ±0.92 [a]	43.62 ±3.13 [c]	56.48 ±0.00 [c]	0 ±0.00 [b]	45.16 ±1.43 [c]	54.80±1.43 [b]	0±0.00 [b]
6	37.15 ± 1.89 [cd]	63.07 ±2.11 [ab]	8.62 ±0.00 [a]	27.69 ±2.71 [d]	72.22 ±2.21 [b]	8.33 ±0.04 [b]	35.71 ±2.87 [c]	64.30±1.78 [b]	6.06±1.09 [b]
8	30.70 ±1.03 [d]	69.67 ±2.33 [a]	9.69 ±0.00 [a]	13.92 ±1.76 [e]	86.11 ±1.61 [a]	11.11 ± 1.82 [a]	22.03 ±1.05 [d]	78.00±1.05 [a]	26.98±1.00 [a]
LSD 0.05	**14.24**	**11.05**	**12.04**	**7.14**	**7.82**	**19.57**	**12.51**	**12.52**	**13.74**

Table (3): LD_{50} and LD_{95} values with their confidence limits for egg, larvae and pupae of *Sitotroga cerealella* exposed to gamma radiation.

Stages	LD_{50}	LD_{95}	Confidence limits(Gy)				**Slope± SD**	**R**
			LD 50		**LTD 95**			
			Lower	Upper	Lower	Upper		
Eggs	170	1630	140	200	1160	2630	1.71± 0.15	0.99
Larvae	60	840	40	800	0.54	1.79	1.47± 0.21	0.99
Pupae	100	1550	70	130	910	3900	1.41± 0. 20	0.98

Table (4): LT_{50} and LT_{95} values with their confidence limits for egg, larvae and pupae of *Sitotroga cerealella* exposed to ozone.

Stages	LT_{50}	LT_{95}	**Confidence limits(h)**				**Slope± SD**	**R**
			LT 50		**LT 95**			
			Lower	Upper	Lower	Upper		
Eggs	3.76	33.08	3.22	4.42	21.69	62.77	1.74± 0.191	0.988
Larvae	2.92	17.45	2.54	3.33	13.15	25.94	2.12± 0.197	0.989
Pupae	3.37	25.24	2.91	3.90	17.63	42.73	1.88± 0. 193	0.989

Effect of ozone and radiation on eggs hatchability %:

As regard to eggs hatchability % of *S. cerealella* exposed as free to ozone and gamma radiation summarized in **Table (5, 6)**. The results showed that, the highest hatchability was recorded 81.33 % (4.69 % reduction) at 1 h exposure period for ozone compared with control (85.33 %), then, significantly decreased with increasing exposure period to 30 % (64.84 % reduction) at 8 h exposure period. Similar results agree with Isikber and Öztekin (2009) that observed higher sensitivity for larvae and pupae of *E. kuehniella* and *T. confusum* to ozone compared to eggs. While, in case of gamma radiation, the highest of eggs hatchability % was 78.67 (7.8 % reduction) at 63 Gy dose compared with control which recorded were 85.33 %. Also, the data significantly decreased gradually as the doses increased to 19.33 % (77.35 % reduction) at 500 Gy dose. Also, the data pointed to that dose of 1000 and 1500 Gy caused a complete inhibition for egg of *S. cerealella*. Hallman (2011) reported that irradiation with at least a 1KGy dose is required for complete disinfestation. The reduction of fertility was dose-dependent and sterility induction could be due to genetic damage (dominant lethal mutations) or physiological damage (reduction in sperm motility or sperm survival in the storage organ) (Sawires, 2005).

Effect of ozone and radiation on wheat germination:

Data in Table (7) revealed insignificant differences in germination of the treated grains with ozone and that untreated, it gave 83 and 77 %, resp. while there is significant decrease in case of radiation. Similar results were found by Geovana (2014), application of O3 treatment only affected wheat germination after 180 min of exposure, reducing germination capacity by 12.5%. Hallman (2011) reported that, irradiation with at least a 1KGy dose is required for complete disinfestation and this would damage most fresh agricultural commodities

Table (5) Hatchability % of *S. cerealella* eggs exposed to gamma radiation

Dose (GY)	N. of hatched Egg	hatchability %	Reduction%
control	42.67±1.77^{a}	85.33±3.53^{a}	-
63	39.33±0.33^{ab}	78.67±0.67^{b}	7.82±0.78^{e}
125	35±1.16^{b}	70±2.31^{c}	17.98±2.71^{d}
250	25.33±1.45^{c}	50.67±1.41^{d}	40.63±3.41^{c}
500	9.67±1.45^{d}	19.33±2.91^{e}	77.35±3.41^{b}
1000	0±0^{e}	0±0^{f}	100±0^{a}
1500	0±0^{e}	0±0^{f}	100±0^{a}
L.S.D.	7.86	6.70	7.86

Table (6) Hatchability % of *S. cerealella* eggs exposed to ozone gas

Exposure time (h)	Number of hatched Egg	Hatchability %	Reduction %
control	42.67±1.77	85.33±3.53^{a}	-
1	40.67±0.88	81.33±1.77^{b}	4.69±2.07^{e}
2	35.33±0.67	70.67±1.33^{c}	17.19±1.56^{d}
4	26.67±0.88	53.33±1.77^{d}	37.50±2.07^{c}
6	20.67±1.20	41.33±2.41^{e}	51.57±2.82^{b}
8	15±1.73	30±3.47^{f}	64.85±4.06^{a}
L.S.D.	8.33	7.15	8.38

Table (7) Germination % of wheat grains treated with ozone and radiation at 8 h exposure period and 1500 Gy. resp

Treatment	No. of germinated grains	Germination %
Ozone (8 hours)	19.25 ±2.07[a]	77 ± 8.30[a]
Radiation (1500 Gy)	2.25 ±1.52[b]	9 ± 6.08[b]
Control	20.75 ±1.32[a]	83 ± 5.24[a]
L.S.D.	4.77	19.10

Effect of ozone and radiation on physical aspects of toast bread:

Data presented in **Table (8)** show that, no significant differences in volume of loaves and specific volume of dough between control samples and those were treated with ozone or radiation. On the other hand, significant decreased in volume of loaves and specific volume in infested flour and treated with radiation compared with infested flour control, meanwhile significant increase in infested flour and treated with ozone.

E**ffect of different treatments on sensory evaluation of toast bread:**

All data in Table (9) showed a significant difference in quality between all treatments. There significant decreased in quality of bread under radiation treatments compare with control fresh on the other hand , in infested wheat there are significant increase in quality bread with ozone or radiation compared with control.

According to the present results, ozone treatments had no effect on milling and baking qualities and also ozone improve quality of bread made from flour infestation with insect. The results agree with the data of Sedlackova *et al* (1986) showed that the irradiation of flour at 0.5 kGy or less generally improve its baking properties, particularly dough development time stability of gelatinization, dough consistency, and increased bread yield and loaf volume. Mendez *et al.* (2003) found that O3 treatment does not significantly change the bread-making properties of the hard wheat, including the tolerance of the dough to over mixing, absorption of water, dough weight, and proof height. Also, Li *et al* (2012) showed that ozone treatment significantly ($P < 0.05$) reduced the microorganisms existed in wheat flour and improved flour and noodle sheet whiteness, dough stability and starch viscosity properties.

Table (8): Effect of different treatments on physical properties (baking quality) of toast bread

Samples	Weight (g)	Volume (cm^3)	Sp.V (cm^3/g)
Control	276.88±1.47 a	625±11.9 a	2.25 a
Ozone	275.48±2.01 a	610.75±4.21 a	2.22 a
radiation	273±1.94 a	597.70±5.01 ab	2.19 a
Control infestation	270.43±0.04 ab	593.75±0.00 b	2.14 ab
Ozone infestation	272.08±0.00 a	598.75±10.2 ab	2.20 a
Radiation infestation	275.50±0.08 a	546.33±0.04 c	1.98 b

Table (9): Sensory evaluation of toast bread prepared with flour exposed to radiation and ozone

Treatments	Characteristics					
	Color of crust (20)	Texture (20)	Taste (20)	Flavor (20)	Appearance (20)	Overall acceptability (20)
Control	18.92±0.34 [a]	17.81±0.32 [a]	16.30±0.42 [a]	17.37 ±0.36 [a]	17.82±0.44 [a]	88.12±0.79 [a]
Ozone	15.50 ±0.26 [bc]	16.3 ±0.36 [b]	15.3 ±0.36 [ab]	15.80 ±0.51 [b]	17.4 ±0.52 [a]	80.30 ±0.90 [b]
radiation	15.70 ±0.42 [bc]	15 ±0.53 [c]	14.6 ±0.30 [bc]	14.82 ±0.38 [bcd]	16.60 ±0.61 [a]	76.70 ±1.19 [c]
Control inf	16.39±0.49 [b]	11.82±0.33 [e]	12.81±0.46 [d]	14.41±0.40 [d]	13.91±0.56 [b]	69.21±0.91 [e]
Ozone inf	15.1±0.27 [c]	14.60±0.37 [c]	13.22±0.44 [d]	15.52±0.40 [bc]	13.61±0.54 [b]	73.01±1.01 [d]
Radiation inf	14.8±0.35 [c]	13.2±0.32 [d]	13.90±0.51 [cd]	15.70±0.51 [bc]	14.3±0.59 [b]	71.9±0.98 [d]
L.S.D.	**1.050**	**1.086**	**1.212**	**1.234**	**1.561**	**2.765**

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ARABIC SUMMARY

تأثير أشعة جاما وغاز الاوزون على أطوار فراشة الحبوب التى تصيب حبوب القمح المخزونة وتأثيرهما على جودة الحبوب

عبد العزيز السيد عبد العزيز، حسن بكرى حسن حسين ، رشا أحمد زينهم
قسم افات الحبوب والمنتجات المخزونة ، معهد بحوث وقاية النباتات ، مركز البحوث الزراعية – الدقى- جيزة – مصر

تهدف هذة الدراسة إلى تقييم فعاليه كل من أشعة جاما بجرعات ٦٣، ١٢٥، ٢٥٠، ٥٠٠، ١٠٠٠، ١٥٠٠ جراي، وغاز الأوزون بتركيز 500 ppm وفترات تعريض ١، ٢، ٤، ٦، ٨ ساعات ضد بيض، ويرقات، وعذارى فراشة الحبوب التى تصيب حبوب القمح المخزنة وكذلك دراسة تأثيرهم على حيوية الحبوب وجودة صفات الخبز.

ويمكن تلخيص النتائج المتحصل عليها فيما يلى :

❖ فى حالة أستخدام أشعة جاما تم الحصول على نسبة أنخفاض كامل فى ذرية الحشرات الناتجة عند جرعة ١٥٠٠ جراى فى جميع الأطوار المعاملة (البيض، اليرقات،العذارى) كما أتضح انه عند تعريض الحبوب الى أشعة جاما كان لها تأثير سلبى على حيوية الحبوب عند جرعة ١٥٠٠ جراى، فى حين كان لها تأثير أيجابى على جودة الخبز عند معاملة القمح المصاب بالأطوار المختلفة لفراشة الحبوب .

❖ أما فى حالة غاز الأوزون وجد أن أقصى نسبة أنخفاض فى ذرية الحشرات الناتجة كانت ٦٩,٦٧% لطور البيض يليه ٧٨ % ، ٨٦,١١ % فى حالة كل من العذارى واليرقات على الترتيب عند التعريض الي ٨ ساعات لغاز الأوزون. كما تأكد أن غاز الأوزون يحسن من حيوية الحبوب وخواص الخبز.

ومن خلال النتائج السابقة أمكن التوصل إلى أن جرعة ١٠٠٠ جراى من أشعة جاما غير كافية للتخلص من طورى البيض والعذارى لفراشة الحبوب.كما أن طور البيض أكثر الأطوار مقاومة لكل من أشعة جاما وغاز الأوزون يليه طور العذراء ثم طور اليرقات التى كانت أكثر حساسية لكل من الأشعاع والأوزون